Electrochemistry on-line with mass spectrometry

Jurva, Johan Ulrik

Summary

The main goal of these studies was the development of new techniques on-line with electrospray mass spectrometry for *in vitro* generation and characterization of drug metabolites. In order to achieve this goal two electrochemical techniques have been developed for oxidation of drugs and xenobiotics on-line with electrospray mass spectrometry.

In chapter 1, a short theoretical introduction is provided to the main concepts discussed in this thesis. The first section gives a historic overview of the developments of electrochemistry coupled on-line with mass spectrometry. The second section gives a short, theoretical description of cytochrome P450, the most important enzyme family in oxidative drug metabolism. The third section provides an introduction to reactive oxygen species (ROS) with the focus on the hydroxyl radical. The ROS section is followed by a brief overview of antioxidants and the chapter is finished with a definition of the scope of the thesis.

In chapter 2, the extent to what electrochemistry on–line with electrospray mass spectrometry (EC/MS) can be used to mimic cytochrome P450 catalyzed oxidations has been investigated. Comparisons at the mechanistic level have been made for most reactions in an effort to explain why certain reactions can, and some cannot, be mimicked by electrochemical oxidations. The EC/MS system provided successful mimics in cases where the P450 catalyzed reactions are supposed to proceed via a mechanism initiated by a one electron oxidation, such as N–dealkylation, S–oxidation, P–oxidation, alcohol oxidation and dehydrogenation. The P450 catalyzed reactions initiated via direct hydrogen atom abstraction, such as O–dealkylation and hydroxylation of aromatic rings without electron donating groups generally had a too high oxidation potential to be electrochemically oxidized below the oxidation potential limit of water, and were not mimicked by the EC/MS system.

In chapter 3, an electrochemical flow through system was introduced that allows the generation of hydroxyl radicals for reaction with xenobiotics and subsequent detection of the oxidation products on–line with LC/MS/MS. The system is based on the Fenton reaction and is predominantly aimed at the generation of hydroxyl radicals, but by minor variations to the system, a broad range of other radicals can be produced. Since the iron is added as Fe^{3+}, the initial mixture is “inactive” until it reaches the electrochemical cell. This makes it very suitable for on–line analysis of the compounds generated, since the whole reaction mixture, including substrate, can be kept in a vial in an autosampler. The system described provides a useful tool for investigation of new radical scavengers and antioxidants. Since the hydroxyl radical adds readily to unsaturated π–systems, the technique is also suitable for on–line generation and characterization of potential drug metabolites resulting from hydroxylation of double bonds and aromatic systems.

In chapter 4, the reactivity of the prenylated flavonoid xanthohumol (XN) towards hydroxyl radicals has been investigated in the electrochemically assisted Fenton system (EC-Fenton system). An experiment with human liver slices was performed and it was found that out of the 10 metabolites showing a mass gain of 16 Da (oxygen), 9 were also formed in the EC-Fenton system. The electrochemically assisted Fenton system provides a valuable tool for investigation of the reactivity of flavonoids towards oxygen radicals. In the case of xanthohumol, the system produces an array of oxidation products that resembles oxidative metabolism in biological systems.

In chapter 5, the two on–line oxidation systems, the EC/MS system and the EC–Fenton system, have been applied to the dopamine agonist S(–)-(N-propyl-N-2-thienylethylamine)-5-hydroxytetralin (N–0923). The oxidative metabolism previously reported from rat liver perfusion experiments was partly mimicked by both methods. The results give an indication that the EC/MS system and the EC–Fenton system give different products and that they can be complementary to each other.

In chapter 6, some of the different problems that one might encounter when coupling an electrochemical cell on-line with an electrospray ionization mass spectrometer are discussed. Some possible solutions are provided for each situation.

In chapter 7, the bioactivation of the potential catecholamine pro–drugs PD217015, (–)-GMC6650 and (+)-GMC6650 has been studied by LC/MS/MS analysis of blood plasma and brain samples from male Wistar rats following oral administration. The catechol metabolite of PD217015, 5,6-dihydroxy-DPAT, was identified in the brain. From interpretation of the product ion spectra, three additional metabolites of PD217015 are suggested. The N–dealkylated metabolite 6-propylamino-3,4,5,6,7,8-hexahydro-2H-naphtalene-1-one and two hydroxylated metabolites suggested to be 2–hydroxy–PD217015 and 4–hydroxy–PD217015. Hydroxylation at position 2 is proposed to constitute an intermediate step in the formation of the active principle 5,6-dihydroxy-DPAT.

For GMC6650, a metabolite believed to correspond to the catechol active principle 1–propyl-1,2,3,4,4a,5,10,10a-octahydro-benzo[g]quinoline-6,7-diol was detected in the brain of the rat given the (–)–enantiomer.

Although MS/MS data often provides useful information about where on a molecule an oxidation has taken place, additional information is sometimes needed to provide sufficient characterization of an unknown metabolite. Both of the electrochemical techniques described above can be scaled up to generate amounts sufficient for analysis with other techniques. If a compound generated with any of the techniques shows the same retention time and product ion spectrum as an unknown metabolite found in a biological sample, a scaled up system could be used to generate this metabolite in amounts sufficient for characterization by IR and NMR.

The simplicity of the techniques, and the ease and speed with which they can be applied to a large number of compounds make them valuable tools in drug metabolism research.